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Action of Monoiodoacetic Acid upon Muscular Function. : Part II. Action of Monoiodoacetic Acid upon Cardiac Muscle.

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Action of Monoiodoacetic Acid upon Muscular Function. : Part II. Action of Monoiodoacetic Acid upon Cardiac Muscle.*

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Abstract

1. The inhibition of the heart beats through vagus nerve stimulation is more pronounced in the poisoned heart muscle than in the normal one. The degree of contraction recovers gradually after the cessation of stimulation, it shows no phase of compensation. 2. The absolute refractory period of the poisoned heart muscle diminishes as the poisoning proceeds.3. The shortening of the absolute refractory period of the poisoned heart muscle through the application of acetylcholin is maintained long after the removal of acetylcholin, though it recovers its previous condition in the normal heart muscle.4. The shortening of the absolute refractory period of the poisoned heart muscle through the vagus nerve stimulation does not recover after the cessation of the stimulation, and if the stimulation be continued longer, the refractory period becomes shorter. 5. In the last stage of poisoning the heart muscle falls spontaneously or by a single stimulation into a peculiar type of contracture.

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Action of Monoiodoacetic Acid upon Muscular Function.
Part II.
Action of Monoiodoacetic Acid upon Cardiac Muscle.

By

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Introduction.

In the previous publication[1], the result of investigation on the condition of the skeletal muscle of R. esculenta poisoned with monoiodoacetic acid from the point of view of muscular activity was reported, and confirmed some peculiar characteristics displayed by a poisoned muscle. So far as the author is aware, no researches have ever been performed on the mechanism of inhibition in muscular activity of a poisoned muscle. Since the mechanism of muscular activity suffers some modifications through the action of monoiodoacetic acid, it is natural to suppose that the inhibitory mechanism, which is another aspect of muscular activity, may also show some changes through its action.

According to *Eggleton* and others[2], the phosphagen content of the cardiac muscle is only one tenth of that of the skeletal muscle, but its significance and physiological role is identical in the skeletal and the cardiac muscles. So the process of poisoning through monoiodoacetic acid in both cases, i.e. the suspension of lactic acid formation, may cause the same results. The object of the present investigation is to find out as to what changes the inhibitory mechanisms of muscular activity may suffer under the action of monoiodoacetic acid. The cardiac muscle, which is innervated by the inhibitory fibres of vagus is best fitted for the purpose of this investigation. So that following experiments were performed to discover the differences of the inhibitory mechanisms of normal and poisoned muscles with reference to vagus stimulation.

Experimental.

I. The effect of vagus stimulation on the frog's heart beats.

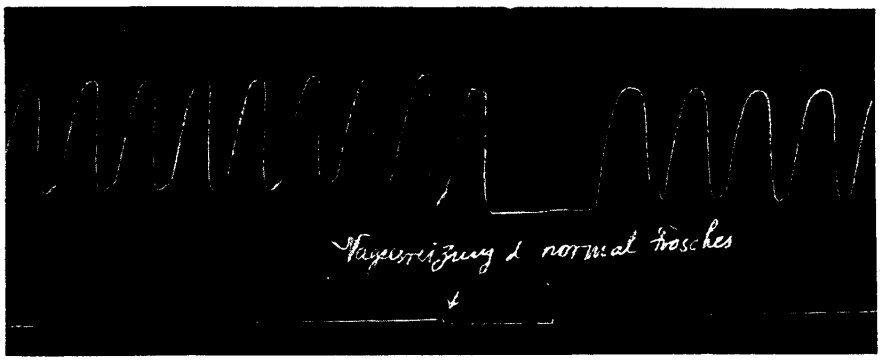
Monoiodoacetic acid solution (0.1% in Ringer) was injected into the hypodermic lymphsack of R. esculenta in the proportion of 1 cc per 1 g of body weight. Thirty minutes after the injection, the heart was exposed and attached to a light lever which registers the heart beats on a rotating smoked drum. At the same time, the vagus nerve was prepared and provided for faradic stimulation. The strength of the stimulus, sufficient to arrest the heart beats was determined beforehand; then the peripheral end of the vagus nerve was placed on a platinum electrode for the purpose of stimulation. Careful observations of the effect on the heart beats were made while stimulation was continued for a suitable length of time.

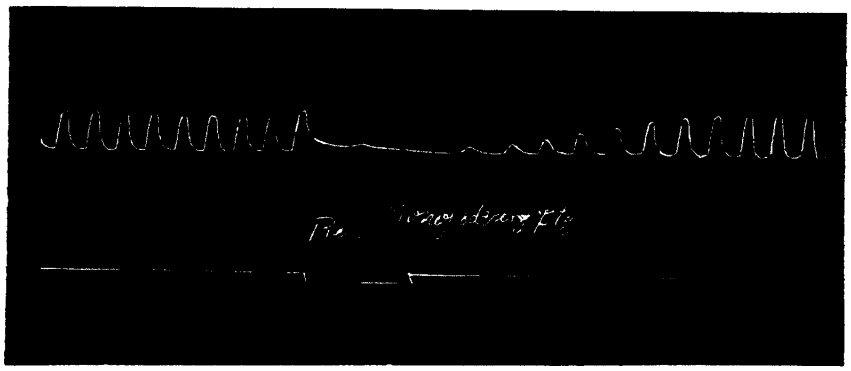
Such operations were repeated on a normal as well as on a poisoned frog's heart. Fig. 1. shows the results obtained. Characteristic differences of the poisoned heart muscle with respect to vagus stimulation are described as follows:

1) The inhibitory effect of vagus nerve stimulation on the beats of the frog's heart is more pronounced than that on a normal one. In other words, the time of the interval between the heart beats compared with that of vagus nerve stimulation is much more marked in a poisoned than in a normal one.

2) When the stimulation is stopped, contrary to the normal case, the heart begins to beat very feebly at first, then recovers gradually and continuously. Compensational augmentation of beats after vagus-inhibition, such as we see always in the normal heart was never observed.

Fig. 1. The effect of vagus stimulation on a frog's heart beats.





II. The refractory period of the poisoned heart muscle.

The principle of determination of the refractory period is the same as described in the previous paper. In the cardiac muscle, the refractory period is much longer than that in the skeletal muscle, so that it was necessary to make some special arrangements to give two successive stimuli with several time intervals. As this arrangement and the direction for use had been previously described by *Hayasi*[3] in our laboratory, and only the more peculiar features of this method will be noted in this paper.

Two contacts whose distance can be varied as desired, are clamped on a vertical shaft and each connected to the primary circuits of two induction coils. These contacts are opened by a falling breaker with constant velocity, by which means two break induction shocks are sent at desired time intervals to the preparat. From the distance of the two contacts which just evokes the summated contraction, the refractory period is calculated in absolute time units. The fallvelocity of the contact breaker is almost uniform and the difference of the distance of the two contacts can be read off on the shaft; 0.5 cm corresponds to 0.32 sec.

Experiments were performed as to the isolated, quiescent, ventricular muscle of frog after making Stannius 2nd ligature. Each of the two stimuli is always the maximum break induction shock. The experimental procedure was undertaken in the following manner.

1) After making Stannius 2nd ligature, the heart was dissected out and tied to a light lever which registers the contraction of the muscle by artificial stimulus on a rotating smoked drum.

2) The coil distance to give the maximum stimulation was determined in each of the two break induction shocks. The electrodes were placed on the apex and the base of the ventricular muscle, and the secondary coils made a common circuit.

3) The least interval for muscular summation was determined by varying the distance of the two contacts. Between each experiment, 1 minute pause was rigorously intercalated by paying attention to the movement of the second-hand of a stop-watch, when it finished one full turn, the heart was lifted up from Ringer's solution in which it had been immersed during the interval. After 10 seconds, the contact breaker begins to fall and opens the two contacts successively. As soon as one experiment ended, the heart was immersed again in Ringer's solution until the next experiment commenced.

During the pause the contact breaker was lifted, and the open contacts were closed and the distance between the two contacts was corrected in accordance with the summated contraction of the muscle obtained in the previous test. The same procedure was repeated until a reliable value was obtained.

4) The determination of the refractory period was performed in two ways; in one experiment the time interval was determined at which ineffective 2nd stimulus became just effective by the gradual increase of the interval of the two stimuli; the second experiment was performed with the opposite object, i.e. determining the interval at which the effective 2nd stimulus becomes just ineffective. The coincidence of these two values was taken as the refractory period.

5) After the absolute refractory period of the normal ventricular muscle was determined, Ringer's solution was replaced by monoiodoacetic acid solution (1/5000 – 1/10000 in Ringer); 10 minutes after the immersion in monoiodoacetic acid solution, the same experiment was repeated and the refractory period of the poisoned cardiac muscle was determined.

6) During the whole series of experiments, all change of the temperature of the solution was avoided as far as possible.

These experimental data are presented in Table 1. In general, the refractory period of the cardiac muscle becomes shorter through the poisoning of monoiodoacetic acid. The action of monoiodoacetic acid is an irreversible one[4], hence it is a matter of regret that the determination could not be undertaken on the heart muscle after recovery from poisoning. Sometimes, in the last stage of poisoning, the heart musle went into a peculiar type of contracture spontaneously or by a single stimulation (see Fig. 3.).

Table 1. The absolute refractory period of the poisoned heart muscle.

No. of Experiments	Temperature (C)	Ringer's solution	MIA-solution (1/10000)	2nd Ringer's solution
1	15.5°	1.98 sec.	> 1.95 sec. >	1.86 sec.
2	17.0°	1.82	> 1.72 =	1.72
3	21.5°	1.02	> 0.90 >	0.87
4	21.0°	1.44	> 1.15 >	0.99
5	13.5°	2.30	< 2.40 >	2.20
6	14.5°	1.73	> 1.70 >	1.57
7	12° – 13°	2.37	< 2.50 >	2.34
8	13.5°	2.24	> 2.17 =	2.17
9	15.0°	2.11	> 1.98 <	2.11
10	19.0°	1.41	> 1.34 =	1.34
11	21.5°	1.18	> 1.02 <	1.09
12	17.0°	1.02	< 1.15 >	0.99
13	17.5°	0.83	> 0.74 >	0.71
14	18.0°	1.54	> 1.34 >	1.28
15	17.5°	1.66	> 1.60 >	1.47

III. The influence of acetylcholin on the refractory period of the cardiac muscle poisoned with monoiodoacetic acid.

Experimental method and arrangements are exactly the same as in the preceeding experiment. First, the refractory period of the normal cardiac muscle was determined. Second, that of the cardiac muscle after immersion in acetylcholin solution (1/20000 in Ringer) was determined. Thirdly, acetylcholin was washed out and replaced by monoiodoacetic acid solution. After 10 minutes, the refractory period of the poisoned cardiac muscle was determined. Sometimes the second and third procedures were reversed.

The results of each experiment agree pretty well (see Table 2. and Fig. 2). It must be acknowledged that the shortening of the refractory period through the action of acetylcholin is maintained after poisoning with monoiodoacetic acid and this effect is not destroyed by washing the muscle.

Table 2. The influence of acetylcholin on the refractory period of the cardiac muscle poisoned with monoiodoacetic acid solution.

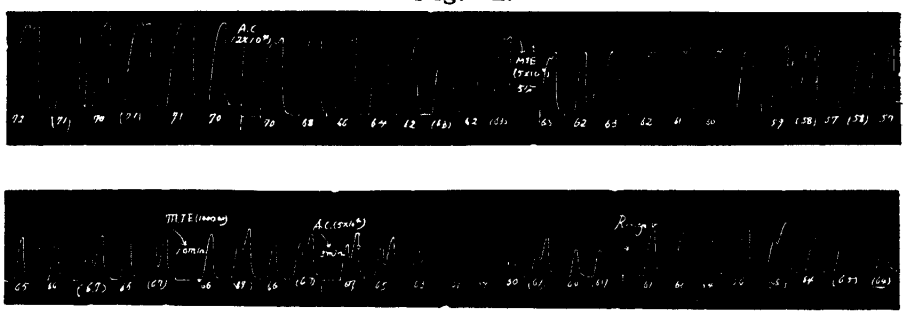
A

No. of Experiments	Temperature (C)	Normal heart muscle in Ringer's solution	Heart muscle in Acetylcholin solution	Poisoned heart muscle in MIA-solution	Poisoned heart muscle in Ringer's solution
1	12.0°	2.62 sec.	> 2.11 sec.	< 1.79 sec.	— sec.
2	12.0°	2.84	> 1.15	< 1.79	> 1.41
3	12.5°	2.50	> 1.54	< 1.73	> 1.67
4	12.0°	3.14	> 2.62	> 2.43	< 2.82
5	11.5°	2.69	> 1.67	< 1.80	= 1.80

B

No. of Experiments	Temperature (C)	Normal heart muscle in Ringer's solution	Poisoned heart muscle in MIA-solution	Poisoned heart muscle in Acetylcholin solution	Poisoned heart muscle in Ringer's solution
1	14.5°	1.98 sec.	> 1.92 sec.	> 1.54 sec.	< 1.86 sec.
2	12.5°	2.37	= 2.37	> 1.99	< 2.18
3	7.0°	3.65	< 3.88	> 3.39	> 2.69
4	9.5°	3.27	> 2.69	= 2.69	—
5	12.5°	2.43	> 2.30	> 1.09	< 1.35

Fig. 2.



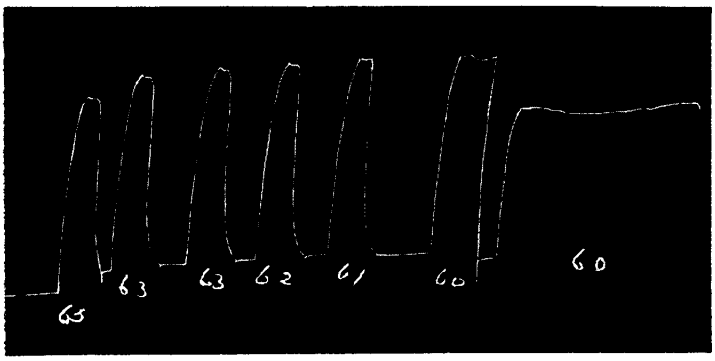
Note: Numbers in the tracing show the intervals of the two successive stimuli.

IV. Influence of vagus stimulation on the refractory period of the cardiac muscle of a tortoise poisoned with monoiodoacetic acid.

Assuming that acetylcholin is *Vagusstuff*[7], it may be supposed that vagus stimulation may affect the refractory period of the cardiac

muscle after poisoning with monoiodoacetic acid in the same way as in the case of the acetylcholin-monoiodoacetic acid application.

Fig. 3. Contracture of the poisoned heart muscle by a single induction shock.



For the purpose of obtaining a non-beating cardiac muscle which has a direct connection with vagus nerve, the ventricular muscle of a tortoise was adopted. As in the former case, Stannius 2nd ligature was made at the auriculo-ventricular groove leaving the fibres of vagus which enter into the ventricular muscle along the hind wall of the heart[5) 6)]. The vagus was prepared for the stimulation in the neck. After the refractory period of the poisoned heart muscle had been determined, stimulation of the vagus was carried out for a short time, viz. 10 secs., then again the refractory period of the cardiac muscle poisoned with monoiodoacetic acid during stimulation of the vagus becomes shorter than that of the non-stimulated one and

Table 3. Influence of vagus nerve stimulation on the refractory period of the cardiac muscle of a tortoise poisoned with monoiodoacetic acid.

No. of Exp.	Normal heart muscle		Vagus stimulation of MIA-heart muscle			
	After Stannius Lig.	Vagus stimulation	1st	2nd	3rd	4th
1	2.18 sec.	1.79 sec.	1.67 sec.	1.60 sec.	1.54 sec.	— sec.
2	3.13	—	2.48	2.21	2.07	1.93
3	3.60	3.30	3.00	2.70	2.47	2.24
4	3.58	3.40	3.29	2.95	2.40	2.35
5	3.87	3.64	3.43	3.08	2.77	2.48
6	2.90	—	2.76	2.34	2.08	—
7	3.56	3.43	3.17	3.02	2.84	2.39

retains the shortened condition of the refractory period, though the normal unpoisoned cardiac muscle recovers from this condition some time after the cessation of stimulation. When the stimulation of vagus is again repeated, the refractory period decreases still further from its already shortened condition. Therefore the effect of stimulation is cumulative. These results are shown in Table 3.

Discussion of the results.

Shortening of the refractory period of the cardiac muscle during the stimulation of its vagus nerve has been confirmed by *Hayasi*[3] in our laboratory. This vagus effect upon the refractory period is still further augmented by the poisoning of cardiac muscle by monoiodoacetic acid not only in the degree of shortening during the stimulation but also in the continued effect after cessation of the stimulation, which have never been observed in the case of unpoisoned cardiac muscle. For the shortening effect of acetylcholin upon the refractory period of cardiac muscle, monoiodoacetic acid acts in exactly the same way as vagus nerve stimulation.

An explanation for this effect may be stated as follow: The inhibitory effect of vagus nerve is nothing but the promotion of the anabolic process of the muscle substance. Acetylcholin is the substance which is liberated at the end of the vagus nerve by stimulation and causes the promotion of the anabolic process of living substances. If it is assumed that the presence of lactic acid retards the progress of the anabolic process of cardiac muscle, then it may be understood why the application of monoiodoacetic acid shortens the refractory period of that muscle.

Summary.

1. The inhibition of the heart beats through vagus nerve stimulation is more pronounced in the poisoned heart muscle than in the normal one. The degree of contraction recovers gradually after the cessation of stimulation, it shows no phase of compensation.

2. The absolute refractory period of the poisoned heart muscle diminishes as the poisoning proceeds.

3. The shortening of the absolute refractory period of the poisoned heart muscle through the application of acetylcholin is maintained long after the removal of acetylcholin, though it recovers its previous condition in the normal heart muscle.

4. The shortening of the absolute refractory period of the poisoned heart muscle through the vagus nerve stimulation does not recover after the cessation of the stimulation, and if the stimulation be continued longer, the refractory period becomes shorter.

5. In the last stage of poisoning the heart muscle falls spontaneously or by a single stimulation into a peculiar type of contracture.

The author wishes to express his thanks to Prof. *S. Oinuma* for his helpful suggestion and advice.

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[1] *Kosaka*, now in press. — [2] *Eggletons*, Journ. of Physiol. Vol. 75, p. 332, 1932. — [3] *Hayasi*, Okayama-Igakkai-Zassi No. 467, p. 2613. — [4] *Lundsgaard*, Biochem. Z. Vol. 227, p. 51, 1930. — [5] *Gaskell*, Journ. of Physiol. Vol. 3, p. 369, 1880–82 & Vol. 4, p. 43, 1883–84. — [6] *Heidenhain*, Pflügers Arch. Vol. 27, p. 383, 1882. — [7] *Yasutake*, Okayama-Igakkai-Zassi No. 456, p. 44.